Bone 54 (2013) 92–97

Contents lists available at SciVerse ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Original Full Length Article

# Low bone mass is prevalent in male-to-female transsexual persons before the start of cross-sex hormonal therapy and gonadectomy☆,☆☆,★,★★

E. Van Caenegem [a,*], Y. Taes [a], K. Wierckx [a], S. Vandewalle [a], K. Toye [a], J.-M. Kaufman [a], T. Schreiner [b], I. Haraldsen [c], G. T'Sjoen [a,d]

[a] Department of Endocrinology, Ghent University Hospital, Ghent, Belgium
[b] Department of Endocrinology, Oslo University Hospital-Rikshospitalet, Oslo, Norway
[c] Department of Neuropsychiatry and Psychosomatic Medicine, Division of Clinical Neuroscience, Oslo University Hospital-Rikshospitalet, Oslo, Norway
[d] Center for Sexology and Gender problems, Ghent University Hospital, Ghent, Belgium

ARTICLE INFO

*Article history:*
Received 25 September 2012
Revised 22 January 2013
Accepted 23 January 2013
Available online 28 January 2013

Edited by: Rene Rizzoli

*Keywords:*
Bone geometry
Transsexuals
Osteoporosis
Body composition

ABSTRACT

*Objective:* Cross-sex hormonal therapy and sex reassignment surgery (including gonadectomy) in transsexual persons has an impact on body composition and bone mass and size. However, it is not clear whether baseline differences in bone and body composition between transsexual persons and controls before cross-sex hormonal therapy play a role.
*Design:* A cross-sectional study with 25 male-to-female transsexual persons (transsexual women) before cross-gender sex steroid exposure (median age 30 years) in comparison with 25 age-matched control men and a male reference population of 941 men.
*Main outcome measures:* Areal and volumetric bone parameters using respectively dual energy X-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT), body composition (DXA), grip strength (hand dynamometer), Baecke physical activity questionnaire, serum testosterone and 25-OH vitamin D.
*Results:* Transsexual women before cross-sex hormonal therapy presented with less muscle mass ($p \leq 0.001$) and strength ($p \leq 0.05$) and a higher prevalence of osteoporosis (16%) with a lower aBMD at the hip, femoral neck, total body (all $p < 0.001$) and lumbar spine ($p = 0.064$) compared with control men. A thinner radial cortex ($p \leq 0.01$) and lower cortical area at the radius and tibia (both $p < 0.05$) was found in transsexual women vs. control men. Serum testosterone was comparable in all 3 groups, but 25-OH vitamin D was lower in transsexual women ($p \leq 0.001$).
*Conclusions:* Transsexual women before the start of hormonal therapy appear to have lower muscle mass and strength and lower bone mass compared with control men. These baseline differences in bone mass might be related to a less active lifestyle.



## Introduction

Male-to-female transsexual persons, also named 'transsexual women', undergo drastic changes in body composition and bone mass due to cross-sex hormonal therapy and sex reassignment surgery (SRS, incl. gonadectomy, vaginoplasty) [1–10]. Up till now, conflicting results have been reported. Most published reports demonstrated a maintained areal bone mineral density (aBMD) after several years of cross-sex hormonal therapy and SRS [3,5,7,12] or even an increase in aBMD after minimum 2 years of treatment [1,6,8–10]. However, a decrease in aBMD in transsexual women using less than 2 years of cross-sex hormonal therapy has been observed [8] and after median 8 years of estrogen therapy transsexual women seemed to have a lower aBMD, volumetric bone mineral density (vBMD) and smaller bone size in relation to lower muscle mass and strength compared with male controls [4,11]. Next to insufficient compliance with estrogen substitution therapy after SRS, another possible reason for the lower aBMD in transsexual women compared with control men, could be the status of bone and body composition before the start of hormonal therapy and SRS. Only one study compared transsexual women before the start of cross-sex hormones and SRS with healthy control men [3]. Other research only described the rate of change of aBMD during hormonal therapy in retrospective [1,6,12] or, prospective [7] design and a few cross-sectional

*Abbreviations:* aBMD, areal bone mineral density; vBMD, volumetric bone mineral density; BMC, bone mineral content; pQCT, peripheral quantitative computed tomography.

☆ Any grants or fellowships supporting the writing of the paper: This work was supported in part by Grant G.0867.11 from the Research Foundation Flanders (FWO Vlaanderen). Eva Van Caenegem and Youri Taes are holders of a PhD fellowship and a postdoctoral fellowship, respectively, from the Research Foundation Flanders.
☆☆ Disclosure summary: The authors have nothing to disclose.
★ Clinical trial registration number: not applicable.
★★ Precis: Transsexual women before the start of hormonal therapy appear to have a lower bone mass and less muscle mass and strength. These baseline differences might be related to a less active lifestyle.

* Corresponding author at: Department of Endocrinology, Ghent University Hospital, De Pintelaan 185, 9000 Ghent, Belgium. Fax: +32 9 332 38 97.
*E-mail address:* eva.vancaenegem@ugent.be (E. Van Caenegem).

8756-3282/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bone.2013.01.039

studies compared transsexual women with control males after 8 months up to 12.5 years of cross-sex hormonal therapy [4,5,9,10]. Haraldsen and colleagues observed a lower aBMD and lean body mass and higher fat body mass in 21 transsexual women prior to hormonal therapy compared with control males [3]. This study and nearly all but two other studies [4,5] used classical dual X-ray absorptiometry (DXA), which has limitations due to its two-dimensional projection technique: i.e. larger bones and projection of soft tissue (i.e. fat mass) can lead to an overestimated aBMD [13].

In this study, we assessed the body composition and bone mass in a group of transsexual women before any kind of hormonal treatment and SRS compared with age-matched control men. In particular, we evaluated both areal bone density using DXA and volumetric bone parameters using peripheral quantitative computed tomography (pQCT).

## Materials and Methods

### *Study Design and Population*

All transsexual participants were diagnosed with gender identity disorder (DSM-IV, 302.85; ICD-10, F64.0) and were recruited from the center for Sexology and Gender Problems at the Ghent University Hospital, Belgium. Every patient was treated in accordance with the World Professional Association for Transgender Health standards of care [14].

This research is part of the 'European network for the investigation of gender incongruence' (ENIGI), a collaboration of four major West European gender identity clinics (Amsterdam, Ghent, Hamburg and Oslo) [15], a study group created to obtain more transparency in diagnostics and treatment of gender identity disorder.

Fifty-eight transsexual women were included in the study. After screening by thorough medical history and determination of serum sex steroids, 8 of them had been using or still used anti-androgens or estrogen substitution therapy. Those participants were excluded from the study. We selected all transsexual women at the age of peak bone mass (between 24 and 46 years old) and compared this group with age-matched control men and with a previously described male reference population, a cohort of young healthy male siblings at the age of peak bone mass ($n = 941$) [16]. Peak bone mass was considered the stable bone mass in men at the ages between 25 and 45 years [17].

A final number of 25 transsexual women who had never used any kind of cross-sex hormonal treatment nor anti-androgen therapy, and thus before SRS, was included. Serum testosterone, SHBG and estradiol levels of the 25 included transsexual women were comparable to that of age-matched control men. All participants were Caucasian. The male control population, matched for age ($\pm 2$ year, median 1 year), were healthy men recruited from suburban communities around Ghent or who responded on posters spread at the Ghent University Hospital and on its website and in schools.

Exclusion criteria for both groups were defined as illnesses or medication use known to affect body composition, hormone levels or bone metabolism such as current, prolonged or previous use (in the last 2 years) of glucocorticosteroids, (anti)androgens, estrogens, calcium and/or vitamin D supplements, antiepileptic drugs, calcitonin, bisphophonates; presence of hypogonadism, untreated hyperthyroidism, cystic fibrosis, malabsorption, eating disorders or disorders of collagen metabolism or bone development, chronic renal failure and autoimmune rheumatoid disease.

All participants were currently in good physical health and completed questionnaires about previous illness and medication use, current and past smoking habits and physical activity by recording the weekly frequency of sports, recreational and/or working activities (using the Baecke questionnaire [18]). Fracture prevalence in the participants was recorded after exclusion of finger, toe, and cranial fractures (a criterion used in previous literature on the male cohort) [19]. Family history on fractures was available by the prevalence of significant fractures in the participants' parents.

The study protocol was approved by the ethics review board of the Ghent University Hospital and all participants gave written informed consent.

### *Body Composition, Muscle Strength and Areal Bone Parameters*

Body weight and anthropometrics were measured in light indoor clothing without shoes. Standing height was measured using a wall-mounted Harpenden stadiometer (Holtain, Ltd., Crymuch, UK).

Grip strength at the dominant hand was measured using an adjustable hand-held standard grip device (JAMAR hand dynamometer, Sammons and Preston, Bolingbrook, IL, USA). The maximum strength of 3 attempts was assumed to best reflect the current status and history of their musculoskeletal adaptation and was expressed in kilograms (kg).

Body fat and lean mass, bone mineral content (BMC), bone area and areal bone mineral density (aBMD) at the lumbar spine and left proximal femur (total hip and femoral neck region) were measured using dual X-ray absorptiometry (DXA) with a Hologic Discovery device (Software Version 11.2.1, Hologic, Inc., Bedford, MA, USA). The coefficient of variation for both spine and whole-body calibration phantoms was less than 1%, as calculated from daily and weekly measurements, respectively.

### *Volumetric Bone Parameters and Cross-sectional Muscle and Fat Area*

A pQCT device (XCT-2000. Stratec Medizintechnik, Pforzheim, Germany) was used to evaluate the cortical volumetric bone parameters at the dominant midradius and tibia (at 66% of bone length) and trabecular bone parameters at the metaphysis (at 4% of bone length) of the dominant radius. Procedure details were as described previously [16].

### *Serum Analysis*

Serum testosterone was determined by liquid chromatography tandem mass spectroscopy (AB Sciex 5500 triple-quadrupole mass spectrometer; AB Sciex, Toronto, Canada) and 25-OH vitamin D by electrochemiluminescence immunoassay, ECLIA (Modular, Roche Diagnostics, Mannheim, Germany). Seasonality was ruled out as all participants were recruited throughout the entire year.

### *Statistical Analysis*

Descriptives are expressed as mean and standard deviation or median [1st to 3rd quartile], when criteria for normal distribution were not fulfilled. *P*-values $< 0.05$ were considered to indicate statistical significance, all tests were two-tailed. Comparison of general characteristics and body composition between groups was made with an independent t-test or Mann–Whitney-U-test when variables were not normally distributed (Table 1). In qualitative variables, chi-square test or, when appropriate, Fisher's exact test was used. Multiple regression analysis was used to compare bone parameters in transsexual women and male controls (Tables 2–4) and used models included height, weight and a grouping variable (transsexual or control group) as independents. The p-value of this grouping variable is indicated in the tables. Correlations were performed using Pearson's correlation coefficient (r) or Spearman's rank correlation coefficient ($r_s$) when variables were not normally distributed. Data were analyzed using SPSS-software, version 19 (SPSS Inc., Chicago, IL).

**Table 1**
Descriptives of general and anthropometric determinations and physical activity in transsexual women before start of cross-gender hormonal treatment.

| | Transsexual women (n = 25) | Matched Control males (n = 25) | Male reference population (n = 941) |
|---|---|---|---|
| Age | 37 (28–42) | 36 (28–42) | 34 (30–39) |
| Weight (kg) | 74.4 ± 15.3 | 77.6 ± 10.7 | 80.8 ± 11.5** |
| Height (cm) | 180.2 ± 5.9 | 181.2 ± 5.8 | 179.6 ± 6.5 |
| Current smoking (%) [a] | 20 | 24 | 23 |
| Pack year (years) | 1 (0–8) | 1 (0–12) | 6 (0–14) * |
| Alcohol intake (units/week) | 0 (0–7) | 15 (5–16)*** | 8 (3–15)*** |
| Fracture prevalence (%) [a] | 20 | 12 | 32 |
| Sport index[b] | 2.5 (1.8–3.5) | 2.8 (2.0–3.8) | 2.8 (2.0–3.3) |
| Leisure time index[b] | 3.0 (2.8–3.3) | 2.8 (2.5–3.3) | 2.8 (2.3–3.0) * |
| Work index[b] | 2.3 (1.9–3.1) | 2.6 (2.3–3.3) | 2.5 (2.1–3.3) |
| Physical activity index[b] | 8.1 (6.5–9.3) | 8.4 (7.9–9.4) | 8.0 (7.3–9.0) |
| Total testosterone (ng/dl) | 587 ± 220 | 578 ± 147 | 584 ± 180 |
| 25-OH vitamin D (ng/ml) | 15 ± 7 | 23 ± 7*** | 21 ± 8** |

Descriptives are expressed as mean ± SD or as median (1st-3rd quartile) when not normally distributed. Variables were compared between groups using independent t-tests or Mann–Whitney-U test when not normally distributed. To convert ng/dl to nmol/l for testosterone multiply by 0.0347. To convert ng/ml to nmol/l for 25-OH vitamin D, multiply by 2.496.
[a] Using chi-square-test.
[b] Measured by the Baecke questionnaire on physical activity [18].
*** $p \leq 0.001$.
** $0.001 < p \leq 0.01$.
* $0.01 < p \leq 0.05$.

**Table 3**
Descriptives of bone parameters as measured by DXA at the lumbar spine and left hip of transsexual women before start of cross-gender hormonal treatment.

| | Transsexual women (n = 25) | Matched Control males (n = 25) | Male reference population (n = 941) |
|---|---|---|---|
| *Lumbar spine* | | | |
| Bone area ($cm^2$) | 69.2 ± 7.0 | 70.7 ± 6.4 | 71.4 ± 6.8* |
| BMC (g) | 67.4 ± 12.4 | 74.2 ± 10.1 | 75.8 ± 13.1*** |
| aBMD ($g/cm^2$) | 0.97 ± 0.13 | 1.05 ± 0.10 | 1.06 ± 0.13* |
| *Femoral neck* | | | |
| Bone Area ($cm^2$) | 5.8 ± 0.5 | 5.7 ± 0.3 | 5.9 ± 0.4 |
| BMC (g) | 4.5 ± 0.8 | 5.2 ± 0.6** | 5.2 ± 0.8** |
| aBMD ($g/cm^2$) | 0.78 ± 0.12 | 0.92 ± 0.13*** | 0.88 ± 0.13** |
| *Total hip* | | | |
| Bone area ($cm^2$) | 44.0 ± 3.8 | 45.2 ± 3.4 | 45.3 ± 4.4 |
| BMC (g) | 41.5 ± 7.9 | 50.1 ± 6.7*** | 48.6 ± 8.1*** |
| aBMD ($g/cm^2$) | 0.94 ± 0.14 | 1.11 ± 0.14*** | 1.07 ± 0.13*** |
| *Whole body* | | | |
| Bone Area ($cm^2$) | 2301.8 ± 160.9 | 2347.9 ± 129.2 | 2354.1 ± 155.1 |
| BMC (g) | 2526.9 ± 326.0 | 2900.4 ± 313.0*** | 2874.0 ± 371.5*** |
| aBMD ($g/cm^2$) | 1.09 ± 0.07 | 1.23 ± 0.09*** | 1.22 ± 0.10*** |

Descriptives are expressed as mean ± SD. All variables were corrected for weight and height.
*** $p \leq 0.001$.
** $0.001 < p \leq 0.01$.
* $0.01 < p \leq 0.05$.

## Results

### *General Characteristics and Physical Activity*

Table 1 summarizes the comparison of general characteristics between transsexual women (n = 25), age-matched controls (n = 25) and male reference population of the same age-range. Body weight, height and BMI were comparable between transsexual women and age-matched control men. There were 20% active smokers in this group compared with 24% controls. Men of the reference population seemed to smoke at a higher rate, as expressed by the higher number of pack years in this group compared with transsexual women. Transsexual women drank less alcohol units per week compared with both control groups. Physical activity during work or leisure time seemed to be higher in the age-matched control group due to a lower work and sport index in transsexual women, but this did not reach the level of significance. Serum 25-OH vitamin D levels were significantly lower in transsexual women compared age-matched control men and the male reference population. Seventy-two percent of transsexual women had a 25-OH vitamin D below 20 ng/ml, versus 32% in age-matched control men and 52% in the male reference population (chi-square test; respectively $p = 0.010$ and $p = 0.065$). Serum

**Table 2**
Descriptives of measures of body composition of transsexual women before start of cross-gender hormonal treatment.

| | Transsexual women (n = 25) | Matched control males (n = 25) | Male reference population (n = 941) |
|---|---|---|---|
| *Measures of fat mass* | | | |
| BMI ($kg/m^2$) | 23.0 ± 4.3 | 23.7 ± 3.2 | 25.1 ± 3.5** |
| Waist circumference (cm) | 82.5 ± 11.0 | 83.7 ± 9.2 | 86.5 ± 9.6 |
| Hip circumference (cm) | 95.2 ± 8.3 | 95.7 ± 5.6 | 96.9 ± 6.7 |
| Waist-to-hip-ratio | 0.86 ± 0.05 | 0.87 ± 0.06 | 0.9 ± 0.1 |
| Fat body mass (kg)[a] | 14.1 ± 6.6 | 13.7 ± 5.6 | 16.2 ± 6.4 |
| Forearm fat CSA ($cm^2$)[b] | 8.0 ± 4.6 | 6.9 ± 3.5 | 8.5 ± 4.1 |
| Calf fat CSA ($cm^2$) [b] | 14.7 ± 6.7 | 13.4 ± 4.5 | 16.7 ± 6.5 |
| *Measures of muscle mass* | | | |
| Grip strength (N/kg) | 44.7 ± 9.0 | 50.1 ± 6.4* | 52.7 ± 7.9*** |
| Lean body mass (kg) [a] | 58.1 ± 9.4 | 61.0 ± 6.8 | 61.7 ± 6.7 |
| Forearm muscle CSA ($cm^2$) [b] | 39.6 ± 6.1 | 45.3 ± 5.9*** | 45.04 ± 6.04*** |
| Calf muscle CSA ($cm^2$) [b] | 78.3 ± 14.1 | 83.4 ± 15.5 | 82.6 ± 11.4 |

CSA: cross-sectional area.
Descriptives are expressed as mean ± SD. Variables were compared between groups using independent t-tests.
[a] Measured with DXA.
[b] Measured with pQCT.
*** $p \leq 0.001$.
** $0.001 < p \leq 0.01$.
* $0.01 < p \leq 0.05$.

**Table 4**
Descriptives volumetric bone parameters as measured by pQCT at the distal (trabecular parameters) and proximal proximal radius and proximal tibia (cortical parameters) of transsexual women before start of cross-gender hormonal treatment.

| | Transsexual women (n = 25) | Matched Control males (n = 25) | Male reference population (n = 941) |
|---|---|---|---|
| *Radius* | | | |
| Trabecular vBMD ($mg/cm^3$) | 218 ± 42 | 244 ± 50* | 229 ± 41 |
| Trabecular bone area ($mm^2$) | 176 ± 22 | 183 ± 28 | 186 ± 26 |
| Cortical vBMD ($mg/cm^3$) | 1091 ± 36 | 1113 ± 28* | 1101 ± 36 |
| Cortical bone area ($mm^2$) | 94 ± 14 | 102 ± 13* | 101 ± 14 |
| Cortical thickness (mm) | 2.21 ± 0.30 | 2.46 ± 0.27** | 2.47 ± 0.33*** |
| Periostal circumference (mm) | 50 ± 4 | 49 ± 4 | 49 ± 4 |
| Endosteal circumference (mm) | 36 ± 5 | 34 ± 4 | 33 ± 5** |
| *Tibia* | | | |
| Cortical vBMD ($mg/cm^3$) | 1120 ± 23 | 1120 ± 28 | 1114 ± 24 |
| Cortical bone area ($mm^2$) | 346 ± 41 | 374 ± 46* | 366 ± 47 |
| Cortical thickness (mm) | 4.24 ± 0.46 | 4.52 ± 0.62 | 4.51 ± 0.55* |
| Periostal circumference (mm) | 95 ± 6 | 97 ± 7 | 95 ± 6 |
| Endosteal circumference (mm) | 69 ± 7 | 69 ± 9 | 67 ± 7 |

Descriptives are expressed as mean ± SD.
All variables were corrected for weight and height. All variables were corrected for weight and height, Logarithmic transformation was used when variables were not normally distributed.
*** $p \leq 0.001$.
** $0.001 < p \leq 0.01$.
* $0.01 < p \leq 0.05$.

testosterone was comparable in all groups. Fracture prevalence in transsexual women was similar to that in both control groups and familial history of fractures was also comparable in all groups (data not shown).

*Body Composition*

Compared to the healthy male reference population(n = 941), transsexual women weighed less and had a lower BMI. Waist and hip circumference and waist-hip ratio were similar in transsexual women and both control groups (Table 2). A considerably lower grip strength and muscle mass, reflected by the muscle cross-sectional area (CSA), was observed at the forearm in transsexual women versus both control groups. Muscle mass at the calf and lean mass also seemed lower in transsexual women compared to both control groups. Serum testosterone was inversely correlated with fat mass in transsexual women (total body fat: $r = -0.423$, $p<0.05$, fat CSA forearm: $r = -0.515$ and fat CSA calf: -0.548, both $p<0.01$).

*Areal Bone Mineral Density Using DXA*

Transsexual women were found to have less bone mineral content (BMC) at the lumbar spine, hip, femoral neck and total body and a smaller bone area at the lumbar spine, total hip and total body than age-matched control men (Table 3). This results in a substantially lower aBMD at all studied sites in transsexual women compared with matched controls. Similar results were observed when compared with the male reference population: transsexual women had lower aBMD and BMC at all measured sites. The prevalence of osteoporosis based on male reference ranges (T-score ≤ 2.5 SD, WHO-criteria as originally proposed for postmenopausal women), was 16% at the lumbar spine in transsexual women versus 4% in the age-matched male controls and 2.1% in the male reference population (chi-square test, respectively non-significant and $p<0.001$). The proportion of transsexual women with osteopenia ranged from 32% at the lumbar spine, 36% at the total hip and up to 44% at the total body and 48% at the femoral neck compared with 4%, 0%, 5 % and 12% respectively of the age-matched control men (chi-square test, all $p \leq 0.004$). Based on female reference ranges to calculate T-score, as more recently suggested to define osteoporosis in men [20], at the lumbar spine, osteoporosis was observed in 8% and osteopenia in 28% of the transsexual women compared with respectively 4% and 12% in age-matched male controls and 0.7% and 14% in the male reference population. A distribution of the z-scores of the lumbar spine is illustrated in Fig. 1, and is different between transsexual women and age-matched control men.

Muscle mass parameters (muscle CSA at the forearm and calf and total body lean mass), were significantly correlated with aBMD and BMC at all measured sites (all $r \geq 0.410$ and $p<0.05$). No significant correlations were observed between areal bone parameters and serum testosterone, 25-OH vitamin D, tobacco use (pack years), alcohol units per week or physical activity in transsexual women.

*Volumetric Bone Parameters at the Upper and Lower Limb Using pQCT*

We observed a significantly lower trabecular volumetric BMD (vBMD) at the distal radius in transsexual women compared to age-matched control men. At cortical sites (proximal radius and tibia), we found a lower cortical bone area and an apparently thinner cortex in transsexual women vs. age-matched controls (Table 4). Compared with the male reference population, the difference in cortical area was less pronounced, but the cortex was significantly thinner in transsexual women probably due to the observed larger endosteal circumference in transsexual women.

Grip strength was positively correlated with trabecular vBMD ($r = 0.478$, $p<0.05$), cortical bone area and thickness at the radius (resp. $r = 0.524$, $p<0.001$ and $r = 0.468$, $p<0.05$). At the tibia, muscle

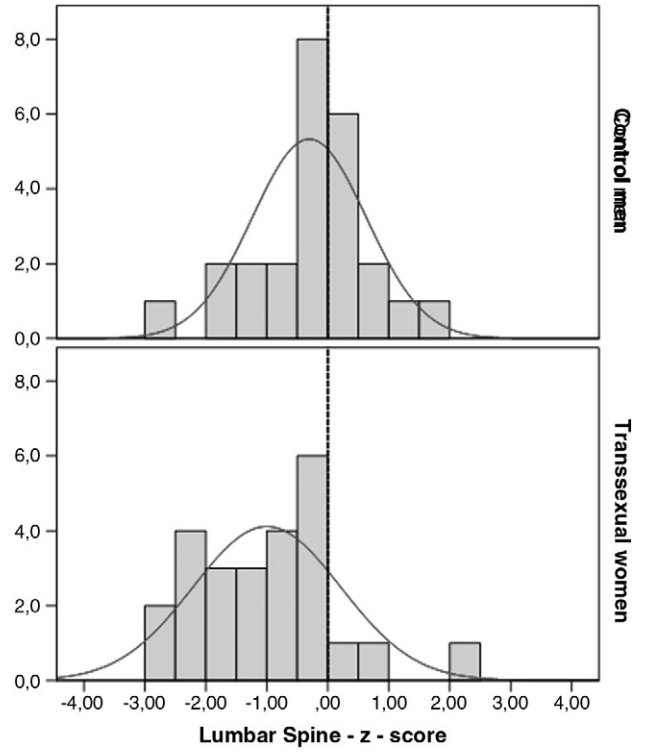


**Fig. 1.** Lumbar spine Z-score distribution in transsexual women before start of cross-gender hormonal treatment compared to control men.

CSA of the calf was positively correlated with cortical bone area and periosteal circumference (resp. $r = 0.527$ and $r = 0.535$, both $p<0.001$). Tibial cortical bone area correlated also with physical activity ($r_s = 0.397$, $p<0.05$). Alcohol units per week were inversely correlated with cortical parameters at the radius in transsexual women (cortical vBMD: $r_s = -0.526$, $p<0.001$, bone area: $r_s = -0.397$ $p<0.05$, cortical thickness: $r_s = -0.433$, $p<0.05$). We found no significant correlations between volumetric bone parameters on the one hand and 25-OH vitamin D, serum testosterone or pack years on the other hand.

## Discussion

To our knowledge, this is the first study to report data on volumetric bone parameters using pQCT in a group of transsexual women prior to any kind of hormonal therapy or gender reassignment surgery. Our results demonstrate that before any kind of cross-sex hormonal treatment or surgery, transsexual women already have a different bone and muscle mass compared with other biological males. Transsexual women present with a lower BMC and aBMD as well as a higher percentage of osteoporosis compared with age-matched control men. Using pQCT, a thinner bone cortex with a lower cortical bone area in transsexual women compared with control men was found. Furthermore, transsexual women before cross-sex hormonal therapy have less muscle mass and strength compared with control men.

Limited data on this subject exists, although our results are in line with the observations of Haraldsen and colleagues (2007), who also found a lower aBMD at the lumbar spine and total hip, lower lean body mass and higher fat mass in 21 Norwegian transsexual women before the start of hormonal therapy [3].

A first explanation for our results can be found in the lower muscle mass and strength in transsexual women. We observed positive associations between muscle mass and strength and areal bone parameters as well as volumetric bone parameters. Indeed, muscle force is an important stimulus for bone growth and maintenance and consequently less mechanical stimuli, due to a lower muscle

mass or lower physical activity, can cause bone loss [21,22]. Transsexual women seemed to participate less in sports and physical activity at work though the results were non-significant. This confirms in previous research in transsexual women [4,23]. It has also been observed that transsexual girls (biological boys) avoided competitive physical sports and were less fond of rough and tumble play [23,24]. We might hypothesize that the lower physical activity, due to different lifestyle of transsexual women, might be one of the reasons for the observed lower muscle mass and strength. In fact, physical activity at young age during growth is mandatory for optimal augmentation of bone mass, cortical bone size and trabecular vBMD, leading to the peak bone mass in young adulthood [25–27]. Later in life, physical activity has been shown to preserve bone mass and structure and might even reduce the risk of osteoporosis [28–31]. In several prospective cohorts of men, a positive relationship between aBMD and physical activity was observed [29,31]. In a Flemish male cohort, at the age of 40, the sports score (Baecke questionnaire) was, after BMI, the most important explaining variable for adult BMD and grip strength was the most important for BMC at the lumbar spine and total body, independent of weight and height [29]. Moreover, higher participation rates in sports (Baecke questionnaire) were associated with higher femoral neck aBMD in 20–49 year old men and women independent of body composition [32]. We could assume that a more sedentary lifestyle, leads to lower aBMD in transsexual women.

A second reason for the lower bone mass in transsexual women might be found in the low vitamin D status of transsexual women. The high prevalence of vitamin D deficiency (72%) in transsexual women might be attributed to a different lifestyle with less social or outdoor activities. Adolescents with gender identity disorder (transsexual girls) participated indeed less in sports, hobbies and games than a reference population [23]. The role of vitamin D in bone health and maintenance is well known [33–38]. Positive relationships have been observed between 25-OH vitamin D and aBMD in several cohorts of young men [34–37] and 25-OH vitamin D is a determinant of peak bone mass [34]. Relationships seem to be strongest when 25-OH vitamin D levels are below 50 nmol/l (20 ng/ml) [33,35,38], which includes the majority of the studied transsexual women. In our group, however, no correlations between 25-OH vitamin D and bone parameters could be observed. Furthermore, vitamin D deficiency is also associated with changes in muscle morphology [34] and even with skeletal muscle function and physical performance, although the evidence for the latter is subject to confounding factors [34,39]. Thus, the lower muscle mass and vitamin D levels could reflect a less active lifestyle of transsexual women, but interaction between these factors could exist.

Thirdly, other lifestyle factors such as alcohol and tobacco use should be considered. The detrimental effects of smoking on bone maintenance [40] and on bone density and geometry in young males are well established [19,41]. High rates of smoking have been reported in transsexual women using hormonal therapy [4,8,42], but in this group of young transsexual women, the percentage of smokers was similar to male controls and the median amount of pack years was even lower. Above that, smoking was not related to bone parameters in this group. The observed difference in bone mass could not be explained by a difference in smoking habits between transsexual women and controls.

The effects of alcohol consumption on bone are linked to the dose ingested and the duration of consumption. From 2 to 4 drinks per day, alcohol can influence bone, although the exact effects depend on the age, sex, and hormonal status of the consumer as well as the type of alcoholic beverage [43]. Transsexual women reported a low weekly alcohol consumption and remarkably lower than control males.

A fourth explanation for the different bone and body composition, can be sought in sex steroid differences between transsexual women and control males. However, the study protocol was performed prior to the start of hormonal therapy and SRS and the use anti-androgens or estrogens in the past was an exclusion criterion. Serum testosterone in transsexual women was comparable to control males. Alterations in testosterone and estrogen have an important effect on bone, but as there are no differences in serum testosterone, sex steroids do not seem to play a role in the lower bone mass of transsexual women. Inverse associations between fat mass parameters and serum testosterone, have also been described in previous research on the male cohort together with a positive relationship between estradiol and fat mass [44]. These associations are most likely due to aromatization of testosterone in fat tissue.

Finally, the lower fracture prevalence in transsexual women may seem in contradiction with the higher prevalence of osteoporosis. This could be due to the lower risk of trauma in transsexual women, as they exhibit a less sportive and less active lifestyle and consequently a lower risk for traumatic fracture [45].

## Conclusion

In conclusion, we found that transsexual women, prior to any hormonal treatment, have lower muscle mass and strength, lower bone mineral density and a smaller cortical bone area and thickness possibly in relation to a different lifestyle compared to control males.

## Acknowledgments

The authors are indebted to Griet De Cuypere, MD, PhD; Gunter Heylens, MD; Els Elaut, MSc, Birgit Van Hoorde, MSc; Steven Weyers, MD, PhD; Piet Hoebeke, MD, PhD; Stan Monstrey, MD, PhD; for referral of participants. We thank all volunteers who participated as study subjects. We thank Jens Jacobeit, MD (Endokrinologikum, Hamburg, Germany) and Mick van Trotsenburg (Vrije Universiteit, Amsterdam, the Netherlands) for their contribution to the development of the ENIGI endocrinological protocol. This work was supported in part by Grant G.0867.11 from the Research Foundation Flanders (FWO Vlaanderen); Eva Van Caenegem and Sara Vandewalle are holders of a PhD fellowship and Youri Taes of a a postdoctoral fellowship from the Research Foundation Flanders.

## References

[1] Dittrich R, Binder H, Cupisti S, Hoffmann I, Beckmann MW, Mueller A. Endocrine treatment of male-to-female transsexuals using gonadotropin-releasing hormone agonist. Exp Clin Endocrinol Diabetes 2005;113:586–92.
[2] Elbers JM, Asscheman H, Seidell JC, Gooren LJ. Effects of sex steroid hormones on regional fat depots as assessed by magnetic resonance imaging in transsexuals. Am J Physiol 1999;276:E317–25.
[3] Haraldsen IR, Haug E, Falch J, Egeland T, Opjordsmoen S. Cross-sex pattern of bone mineral density in early onset gender identity disorder. Horm Behav 2007;52:334–43.
[4] Lapauw B, Taes Y, Simoens S, Van Caenegem E, Weyers S, Goemaere S, et al. Body composition, volumetric and areal bone parameters in male-to-female transsexual persons. Bone 2008;43:1016–21.
[5] Lips P, Asscheman H, Uitewaal P, Netelenbos JC, Gooren L. The effect of cross-gender hormonal treatment on bone metabolism in male-to-female transsexuals. J Bone Miner Res 1989;4:657–62.
[6] Mueller A, Dittrich R, Binder H, Kuehnel W, Maltaris T, Hoffmann I, et al. High dose estrogen treatment increases bone mineral density in male-to-female transsexuals receiving gonadotropin-releasing hormone agonist in the absence of testosterone. Eur J Endocrinol 2005;153:107–13.
[7] Mueller A, Zollver H, Kronawitter D, Oppelt PG, Claassen T, Hoffmann I, et al. Body composition and bone mineral density in male-to-female transsexuals during cross-sex hormone therapy using gonadotrophin-releasing hormone agonist. Exp Clin Endocrinol Diabetes 2011;119:95–100.
[8] Reutrakul S, Ongphiphadhanakul B, Piaseu N, Krittiyawong S, Chanprasertyothin S, Bunnag P, et al. The effects of oestrogen exposure on bone mass in male to female transsexuals. Clin Endocrinol (Oxf) 1998;49:811–4.
[9] Ruetsche AG, Kneubuehl R, Birkhaeuser MH, Lippuner K. Cortical and trabecular bone mineral density in transsexuals after long-term cross-sex hormonal treatment: a cross-sectional study. Osteoporos Int 2005;16:791–8.
[10] Sosa M, Jodar E, Arbelo E, Dominguez C, Saavedra P, Torres A, et al. Bone mass, bone turnover, vitamin D, and estrogen receptor gene polymorphisms in male to female transsexuals: effects of estrogenic treatment on bone metabolism of the male. J Clin Densitom 2003;6:297–304.

[11] T'Sjoen G, Weyers S, Taes Y, Lapauw B, Toye K, Goemaere S, et al. Prevalence of low bone mass in relation to estrogen treatment and body composition in male-to-female transsexual persons. J Clin Densitom 2009;12:306–13.
[12] Van Kesteren P, Lips P, Gooren LJ, Asscheman H, Megens J. Long-term follow-up of bone mineral density and bone metabolism in transsexuals treated with cross-sex hormones. Clin Endocrinol (Oxf) 1998;48:347–54.
[13] Carter DR, Bouxsein ML, Marcus R. New approaches for interpreting projected bone densitometry data. J Bone Miner Res 1992;7:137–45.
[14] World Professional Association for Transgender Health Standards, WPATH. Standards of Care for the Health of Transsexual, Transgender, and Gender Nonconforming People 7th version. http://www.wpath.org/publications_standards.cfm; 2012.
[15] Kreukels BP, Haraldsen IR, De CG, Richter-Appelt H, Gijs L, Cohen-Kettenis PT. A European network for the investigation of gender incongruence: the ENIGI initiative. Eur Psychiatry 2012;27:445–50.
[16] Lapauw B, Taes Y, Bogaert V, Vanbillemont G, Goemaere S, Zmierczak HG, et al. Serum estradiol and not testosterone influences volumetric bone mineral density and modulates the impact of physical activity on bone size at the age of peak bone mass — a study in healthy male siblings. J Bone Miner Res 2009;24:1075–85.
[17] Heaney RP, Abrams S, Dawson-Hughes B, Looker A, Marcus R, Matkovic V, et al. Peak bone mass. Osteoporos Int 2000;11:985–1009.
[18] Baecke JA, Burema J, Frijters JE. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. Am J Clin Nutr 1982;36:936–42.
[19] Taes Y, Lapauw B, Vanbillemont G, Bogaert V, De Bacquer D, Goemaere S, et al. Early smoking is associated with peak bone mass and prevalent fractures in young, healthy men. J Bone Miner Res 2010;25:379–87.
[20] Kanis JA, Bianchi G, Bilezikian JP, Kaufman JM, Khosla S, Orwoll E, et al. Towards a diagnostic and therapeutic consensus in male osteoporosis. Osteoporos Int 2011;22:2789–98.
[21] Cheung AM, Giangregorio L. Mechanical stimuli and bone health: what is the evidence? Curr Opin Rheumatol 2012;24:561–6.
[22] Frost HM. Bone "mass" and the "mechanostat": a proposal. Anat Rec 1987;219:1–9.
[23] Cohen-Kettenis PT, Owen A, Kaijser VG, Bradley SJ, Zucker KJ. Demographic characteristics, social competence, and behavior problems in children with gender identity disorder: a cross-national, cross-clinic comparative analysis. J Abnorm Child Psychol 2003;31:41–53.
[24] Zucker KJ. Measurement of psychosexual differentiation. Arch Sex Behav 2005;34:375–88.
[25] Gunter KB, Almstedt HC, Janz KF. Physical activity in childhood may be the key to optimizing lifespan skeletal health. Exerc Sport Sci Rev 2012;40:13–21.
[26] Lorentzon M, Mellstrom D, Ohlsson C. Association of amount of physical activity with cortical bone size and trabecular volumetric BMD in young adult men: the GOOD study. J Bone Miner Res 2005;20:1936–43.
[27] Nilsson M, Ohlsson C, Sundh D, Mellstrom D, Lorentzon M. Association of physical activity with trabecular microstructure and cortical bone at distal tibia and radius in young adult men. J Clin Endocrinol Metab 2010;95:2917–26.
[28] Cousins JM, Petit MA, Paudel ML, Taylor BC, Hughes JM, Cauley JA, et al. Muscle power and physical activity are associated with bone strength in older men: the osteoporotic fractures in men study. Bone 2010;47:205–11.
[29] Delvaux K, Lefevre J, Philippaerts R, Dequeker J, Thomis M, Vanreusel B, et al. Bone mass and lifetime physical activity in Flemish males: a 27-year follow-up study. Med Sci Sports Exerc 2001;33:1868–75.
[30] Kohrt WM, Bloomfield SA, Little KD, Nelson ME, Yingling VR. American College of Sports Medicine Position Stand: physical activity and bone health. Med Sci Sports Exerc 2004;36:1985–96.
[31] Morseth B, Emaus N, Wilsgaard T, Jacobsen BK, Jorgensen L. Leisure time physical activity in adulthood is positively associated with bone mineral density 22 years later. The Tromso study. Eur J Epidemiol 2010;25:325–31.
[32] Camhi SM, Katzmarzyk PT. Total and femoral neck bone mineral density and physical activity in a sample of men and women. Appl Physiol Nutr Metab 2012;37:947–54.
[33] Lips P. Vitamin D, deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. Endocr Rev 2001;22:477–501.
[34] Boot AM, Krenning EP, de Muinck Keizer-Schrama SM. The relation between 25-hydroxyvitamin D with peak bone mineral density and body composition in healthy young adults. J Pediatr Endocrinol Metab 2011;24:355–60.
[35] Frost M, Abrahamsen B, Nielsen TL, Hagen C, Andersen M, Brixen K. Vitamin D status and PTH in young men: a cross-sectional study on associations with bone mineral density, body composition and glucose metabolism. Clin Endocrinol (Oxf) 2010;73:573–80.
[36] Lim JS, Kim KM, Rhee Y, Lim SK. Gender-dependent skeletal effects of vitamin D deficiency in a younger generation. J Clin Endocrinol Metab 2012;97:1995–2004.
[37] Szulc P, Munoz F, Marchand F, Chapuy MC, Delmas PD. Role of vitamin D and parathyroid hormone in the regulation of bone turnover and bone mass in men: the MINOS study. Calcif Tissue Int 2003;73:520–30.
[38] Ooms ME, Lips P, Roos JC, van der Vijgh WJF, Popp-Snijders C, Bezemer PD, et al. Vitamin D status and sex hormone binding globulin: determinants of bone turnover and bone mineral density in elderly women. J Bone Miner Res 1995;10:1177–84.
[39] Girgis CM, Clifton-Bligh RJ, Hamrick MW, Holick MF, Gunton JE. The roles of vitamin d in skeletal muscle: form, function, and metabolism. Endocr Rev 2013;34. http://dx.doi.org/10.1210/er.2012-1012.
[40] Kanis JA, Johnell O, Oden A, Johansson H, De Laet C, Eisman JA, et al. Smoking and fracture risk: a meta-analysis. Osteoporos Int 2005;16:155–62.
[41] Rudäng R. Smoking is associated with impaired bone mass development in young adult men: a 5-year longitudinal study. J Bone Miner Res 2012;27:2189–97.
[42] Wierckx K, Mueller S, Weyers S, Van Caenegem E, Roef G, Heylens G, et al. Long-term evaluation of cross-sex hormone treatment in transsexual persons. J Sex Med 2012;9:2641–51.
[43] Maurel DB, Boisseau N, Benhamou CL, Jaffre C. Alcohol and bone: review of dose effects and mechanisms. Osteoporos Int 2012;23:1–16.
[44] Vanbillemont G, Lapauw B, Bogaert V, De Naeyer H, De Bacquer D, Ruige J, et al. Birth weight in relation to sex steroid status and body composition in young healthy male siblings. J Clin Endocrinol Metab 2010;95:1587–94.
[45] Schalamon J, Dampf S, Singer G, Ainoedhofer H, Petnehazy T, Hoellwarth ME, et al. Evaluation of fractures in children and adolescents in a Level I Trauma Center in Austria. J Trauma 2011;71:E19–25.